FURTHER STUDIES ON THE PRODUCTION OF FUNCTIONAL AND RUDIMENTARY SPERMATOZOA IN ROTIFERS.[1]

D. D. WHITNEY.

In a recent paper some general observations were recorded in regard to the dimorphic spermatozoa found in the parthenogenetically developed males of nine species of rotifers. Some of the stages in the development of the spermatozoa from the cells of the last spermatocyte-divisions in *Brachionus mulleri* were described and figured. No studies, however, were made on the nuclear material of the spermatozoa either to determine whether it was present in both kinds of spermatozoa or to determine in what part of the spermatozoa it was located.

Additional studies now have been completed in an attempt to observe these points. These studies have been made in considerable detail on the marine species, *Brachionus mulleri*, and in lesser detail on *Hydatina senta*, *Brachionus amphiceros* and *Diaschiza sterea*. As the earlier paper had some of the details of the formation of the spermatozoa of *Brachionus mulleri* and as this rotifer is one of the most convenient forms to maintain in the laboratory and also a very convenient one from which to obtain immature males the majority of the observations were made on material from this species.

Several killing and fixing fluids and stains were tried but Zenker's fluid followed by Delafield's hematoxylin proved to be the most satisfactory method for making clear mounts of the entire cells. The living males were crushed under a cover glass in culture water and favorable cells were selected under the microscope. The killing fluid was allowed to run under the cover glass and to remain from 10-20 minutes. Later the killing fluid was drawn out by filter paper and distilled water allowed to take its place. After the distilled water had been changed several times from under the cover glass Delafield's hematoxylin

[1] Studies from the Zoölogical Laboratory, The University of Nebraska, No. 119.

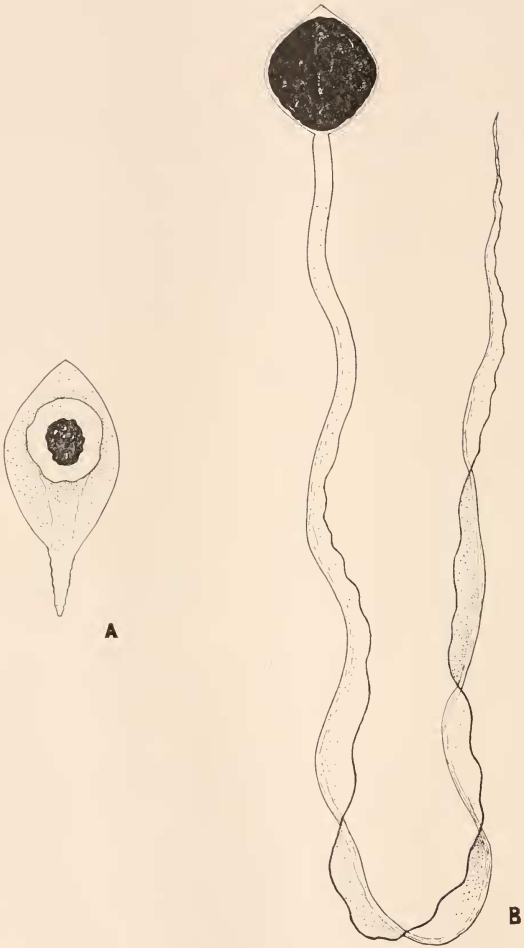


FIG. 1. *Brachionus mulleri*. *A*, normal spermatid showing the nucleus, chromatin, and the small tail; *B*, mature and normal spermatozoön showing the large chromatin mass in the head and the long vibratile tail with the undulating membrane. (Drawn by measurements from living and stained specimens with 1/12 oil immersion objective and a number 2 ocular.)

(diluted 1:1) was allowed to run under and to stain the cells for about two minutes. This was then washed out with tap water. All of these processes were carried on under the microscope in order to make certain that no mistakes of indentification of these cells should be made after the slides were finished. Although this is a tedious method it has the advantage of being perfectly reliable and furnishing accurate data concerning which there can be no doubts. Many other methods were tried but none were found to be as practical and as certain as this one.

The nucleus was first indentified in an early stage of the normal spermatid (Fig. 1, *A*) which later would have developed into the large motile spermatozoon (Fig. 1, *B*).

The degenerate sperm cells from the same male individual were examined next and each was found to contain a nucleus with chromatin material in it. This stage is shown in Fig. 2, *A*. This fact is of considerable interest because it demonstrates that in the division that forms the secondary spermatocytes there is evidently a division of the chromatin material and consequently all the secondary spermatocytes contain some of this chromatin material. One half of these secondary spermatocytes divide and form the normal spermatids but the remaining half of the secondary spermatocytes do not divide again but develop directly into the degernerate spermatozoa. The early stage of these degenerate spermatocyte cells are smaller in size than the normal cells of the same age but the nucleus is only slightly smaller than the nucleus contained in the normal spermatid cell as can be seen by comparing Figs. 2, *A* and 1, *A*.

The later development of each of these two kinds of cells is very different. In the normal cell the nucleus grows larger and larger until in its final stage it is several times its former size. From one end of the cell an outgrowth appears which grows longer and longer and finally becomes vibratile. This is the motile tail of the sperm cell. It is very long and large and has an undulating membrane along the greater portion of the dorsal side. Fig. 1, *B*, shows the entire matured spermatozoön containing the large mass of chromatin material in the head.

The development of the smaller and degenerate spermatocyte cells into the complete rudimentary spermatozoa is strikingly

different from the development of the normal spermatid cells. After the early stage is passed the chromatin material increases in bulk very slightly and becomes composed of coarse granules

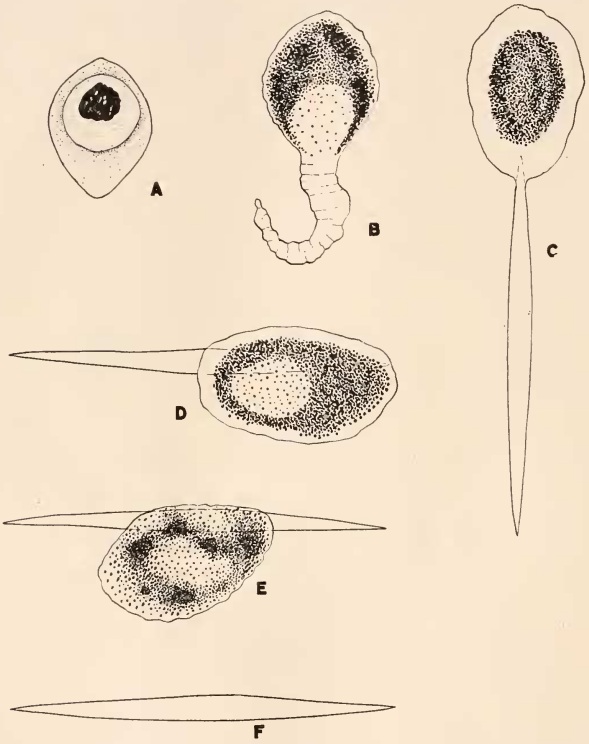


FIG. 2. *Brachionus mulleri*. *A*, early stage in the development of the rudimentary spermatozoön showing the chromatin in the nucleus. This cell is from the same male as the normal spermatid of Fig. 1, *A*, and is of the same age. *B*, a later stage showing the tail wrinkled and distorted by the fixing fluid; *C*, last stage showing the stiff and rigid tail attached to the head which contains the chromatin; *D*, the stiff tail forced through the chromatin to the opposite side of the head by pressure under the cover glass; *E*, the stiff tail forced through the opposite wall of the head by pressure under the cover glass; *F*, the stiff tail detached from the head. (Drawn to same scale as Fig. 1.)

loosely arranged and persists in this form throughout the remainder of the development of the cell. From one end of the cell a process extends which is somewhat flexible in its earlier development. Fig. 2, *B*, shows such an early stage in which the tail-like process has become curved and wrinkled after treatment with Zenker's fluid. Later this tail-like process becomes very rigid and stiff. So stiff that when the immature males are crushed this process by chance may be driven through the nuclear material to the opposite side of the cell as shown in Fig. 2, *C*, or in some instances it was even driven through the opposite wall of the cell as shown in Fig. 2, *D*. Normally this stiff process seems to be attached to one end of the cell. Later, however, when it is fully formed it becomes detached from the cell. This separation of the two parts of the cell occurs in the testis either before the male hatches from the egg or very soon thereafter. Fig. 2, *C*, shows the two parts just prior to separation.

This stiff spindle-shaped part contains no chromatin material and probably is to be considered the rudimentary tail of the cell. It is immotile and much smaller than the tail of the motile spermatozoön. As it separates from the nucleated portion of the cell and is immotile one could naturally conclude that it is to be regarded as simply a degenerate or rudimentary tail which has no function. Whether the nucleated portion of the cell has any function is unknown but as it is also immotile it is also very probably functionless.

In the earlier studies the detached tails of the normal spermatozoa were erroneously considered to be the entire spermatozoa. When the males were crushed under a cover glass these tails were extruded and were able to move about in the water for a considerable length of time. When recently, however, these so-called spermatozoa were stained no nucleus or chromatin material could be found in any of them. As they are the functional spermatozoa it was realized that there must be a nucleus and chromatin material somewhere in them.

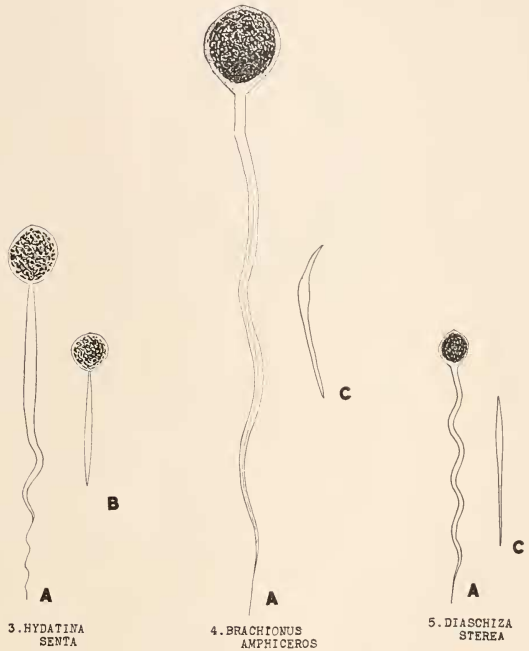
It was recalled that in previous observations some males had been seen to extrude a few of these motile bodies which had a large swelling on the anterior end. At that time these motile bodies were considered to be the immature spermatozoa. When

more careful observations were made both upon such living cells and upon stained ones it was readily seen that these cells were not immature stages but were the mature stages of the spermatozoa. The heads are quite large in proportion to the diameter of the tail, Fig. 1, *B*, and are easily separated from the tails. In the testis of the male these spermatozoa are in clusters and in each cluster each spermatozoön is attached to some sort of a central tissue in the cluster. Normally in copulation with the young females a few of these spermatozoa become detached from the central tissue and are extruded into the body cavity of the female. When, however, the males are crushed under a cover glass nearly every head remains attached to this central tissue but the tails break off from the heads and are immediately extruded in a writhing mass. If the males are sufficiently crushed the mass of spermatozoa heads will also be extruded together with other cells of the broken-down tissues. If very young males are taken from the eggs and are crushed all of the immature sperm cells with the tails attached will be extruded. The ripe spermatozoa resemble very closely these immature spermatozoa except their heads are more rounded and the tails are somewhat longer.

The males of another rotifer, *Hydatina senta*, were crushed and the spermatozoa reëxamined. A few males shed both kinds of spermatozoa with the tails attached to the heads. These were stained and the heads of each kind were found to contain chromatin material similar to the heads of the spermatozoa of *Brachionus mulleri*. These are shown in Fig. 3. Twenty-five males were crushed and out of the many hundred spermatozoa ejected only about 6–8 entire spermatozoa of each kind with the tail attached to the head were found. *Hydatina senta* males are the most favorable material thus far examined in which to see the two kinds of complete spermatozoa in a mature male. In *Brachionus mulleri* these two kinds of complete spermatozoa could only be found in the unhatched and immature males.

The spermatozoa were examined from mature males of two additional species of rotifers, *Brachionus amphiceros*, and *Diaschiza sterea*. The entire normal spermatozoa with the tails attached to the heads were found in each species. Upon staining

the heads showed their chromatin contents. Only the tails of the rudimentary spermatozoa were found and they showed no chromatin material in them when stained. Sketches of these spermatozoa and tails are shown in Figs. 4 and 5.



FIGS. 3–5. Dimorphic spermatozoa and tails of three other species of rotifers. *A*, larger normal and motile spermatozoa showing the head and the contained chromatin; *B*, smaller, rudimentary and non-motile spermatozoön showing the chromatin in the head; *C*, detached tails of rudimentary spermatozoa. (Drawn with 4 mm. objective and number 2 ocular.)

The spermatozoa of the other species of rotifers enumerated in the former paper have not been reëxamined because these forms are not available at this season of the year.

As the stained slides of the sectioned unlayed fertilized eggs of *Hydatina senta* were available for study they were examined to

determine the size and condition of the head of the spermatozoön after it had entered the egg. The egg examined was sectioned while inside the body of the female and although it had the thick secretion around it which later would have shrunken to form the heavy external covering or shell it was readily sectioned at this

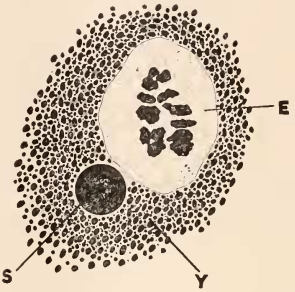


FIG. 6. Portion of a fertilized egg of *Hydatina senta*. *E*, egg nucleus containing chromosomes; *S*, sperm-head; *Y*, yolk granules of the egg. (Sperm-head and the outline of egg nucleus drawn to same scale as in Figs. 3–5. Yolk granules and chromosomes drawn freehand without measurements.)

early stage. The sperm head is seen near the nucleus of the egg (Fig. 6). It is about the size of the head of the normal spermatozoön as can be seen by comparing it with Fig. 3, but contains denser chromatin material and is perfectly round in form.

Dimorphic spermatozoa have been studied in considerable detail in some of the Mollusca and in some of the Lepidoptera by several workers. Among these Goldschmidt and Gatenby recently have come to the conclusion that the atypical or abnormal spermatozoa are wholly functionless and the latter is of the opinion that such spermatozoa have no significance in regard to sex regulation. According to their conclusion any spermatozoa and in some cases all spermatozoa of these forms studied may become degenerate if the male individual is in a certain physiological condition.

In the phylloxerans and in the rotifers the case is quite different. In each and every male a certain percentage of the sperm

cells degenerate and a certain percentage of the sperm cells develop normally, thus giving a definite ratio of degenerate and normal sperm cells. In the phylloxerans this ratio has not been determined but in the rotifers there is one degenerate sperm cell to two normal sperm cells. As all the fertilized eggs in both phylloxerans and rotifers develop into female young it seems safe to conclude, as Morgan has already concluded, that the degenerate sperm cells are the male-determining ones and that the normal sperm cells are the female-determining ones.

Summary.

1. The normal and motile spermatozoa of *Brachionus mulleri*, *Brachionus amphiceros*, *Hydatina senta* and *Diaschiza sterea* possess large heads in which is located the chromatin material.

2. The motile bodies of seven other species of rotifers which were figured and considered as the normal spermatozoa in the former paper were probably only the tails of such normal spermatozoa.

3. The degenerate and immotile spermatozoa as seen in *Brachionus mulleri* and *Hydatina senta* also possess heads in which there is chromatin material.

4. The stiff processes, called rudimentary spermatozoa in the former paper, are now considered to be not the complete rudimentary spermatozoa but only the degenerate tails of these rudimentary spermatozoa.

5. The sperm head in the fertilized egg of *Hydatina senta* is about the same size as the head of the normal spermatozoön of this species.

6. The four species of rotifers enumerated above together with the seven other species described in the former paper constitute eleven species of rotifers in which both normal and rudimentary spermatozoa have been found to occur.

Zoölogical Laboratories,
The University of Nebraska,
Lincoln, Nebraska, January 5, 1918.

Literature.

Federly, H.

'13 Das Verhalten der Chromosomen bei der Spermatogenese der Schmetterlenge, etc. Zeit. f. unduktive Abstam. Vererb., Bd. 9.

Gatenby, J. Bronté

'17 The Degenerate (Apyrene) Sperm-formation of Moths as an Index to the Inter-relationship of the Various Bodies of the Spermatozoon. Quart. Journ. Micro. Sci., No. 247, Vol. 62.

Goldschmidt, Richard

'16 The Function of the Apyrene Spermatozoa. Science, No. 1137, Vol. 44.

Kuschakewitsch, S.

'13 Studien uber den Polymorphisms der mannlichen Geschlechtselemente bei den Prosobranchia. Arch. Zellforschg, Vol. 10 (complete literature).

Meves, Fr.

'03 Ueber oligopyrene und apyrene Spermien und ueber ihre Entstehung nach Beobachtungen au Paludina und Pygaera. Arch. Mikr. Anat., Vol. 61. (Reference to previous literature.)

Morgan, T. H.

'09 A Biological and Cytological Study of Sex Determination in Phylloxerans and Aphids. Journ. Exper. Zoöl., Vol. 7.

Reinke, E. E.

'14 The Development of the Apyrene Spermatozoa of Strombus bituberculatus. Tortugas Laboratory, Vol. 6.

Whitney, D. D.

'13 An Explanation of the Non-Production of Fertilized Eggs by Adult Male-Producing Females in a Species of Asplanchna. BIOL. BULL., Vol. 25.

'17 The Production of Functional and Rudimentary Spermatozoa in Rotifers. BIOL. BULL., Vol. 33.